Phylogeny of Philippine slender skinks (Scincidae: Brachymeles) reveals underestimated species diversity, complex biogeographical relationships, and cryptic patterns of lineage diversification

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1. Introduction

Only four genera of scincid lizards are known to possess both fully limbed and limbless species (Brachymeles, Chalcides, Lerista, and Scelotes; Lande, 1978; Wiens and Slingsluff, 2001; Brandley et al., 2008). Of these four genera, the genus Brachymeles is the least well known, with recent studies indicating the recognized diversity of the group is vastly underestimated (Siler et al., 2009, 2010a,b, in press; Siler and Brown, 2010). Within the genus, all but one of the 25 recognized species are endemic to the Philippines (Brown, 1956; Brown and Rabor, 1967; Brown and Alcala, 1980; Siler et al., 2009, 2010a,b; Siler and Brown, 2010; Siler et al., in press); the exception is B. apus from northern Borneo (Hikida, 1982). Thirteen species are pentadactyl (biolorb, boholensis, boulenegi, gracilis, sp. A [Masbate Island; Siler and Brown, 2010], makosog, mindorensis, orientalis, schadenbergi, talinis, taylori, sp. B [Luzon + Babuyan islands; Siler and Brown, 2010], and sp. C [Jolo Island; Siler and Brown, 2010]), eight are non-pentadactyl, with incompletely developed limbs and reduced numbers of digits (boni, cebuensis, elerae, muntingkayam, pathfinderi, samarensis, tridactylus, and wrighti), and four are entirely limbless (apus, miminus, lukbani, and vermis). Within the non-pentadactyl species, there exist a wide range of limb- and digit-reduced states. Some species have minute limbs that lack full digits (boni, cebuensis, muntingkayam, samarensis, tridactylus; Duméril and Bibron, 1839; Brown, 1956; Brown and Rabor, 1967; Siler et al., 2009). Other non-pentadactyl species have moderately developed limbs with four digits on the hands and feet (elerae, wrighti), or four digits on the feet and five digits on the hands (pathfinderi; Taylor, 1917, 1925). All species are semi-fossorial and typically found in dry, rotting material inside decaying logs or in loose soil, forest floor detritus, and leaf litter.

The genus Brachymeles was first described by Duméril and Bibron (1839) for the small, limb-reduced species Brachymeles...
As species diversity has accumulated, various authors have noted morphological variation among island populations of many of the polytypic and widespread species (Taylor, 1922; Brown, 1956; Brown and Rabor, 1967; Brown and Alcala, 1980). In Brachymeles, several species (B. Bonitae, B. samarensis, B. tridactylus) currently span recognized faunal regions within the Philippines, or Pleistocene Aggregate Island Complexes (PAICs; Brown and Guttman, 2002; Brown and Diesmos, 2002, 2009; Fig. 1, and defy biogeographic boundaries as traditionally conceived (Brown and Diesmos, 2009). Among skinks and other Philippine land vertebrates, multiple lineages have similar widespread distributions, spanning multiple PAICs. These widespread distributions have been the focus of many recent studies, which have revealed that few endemic Philippine reptiles actually possess broad distributions spanning regional faunistic boundaries (Brown et al., 2000, 2009; Brown and Diesmos, 2002, 2009; Siler et al., 2010a,b; Siler and Brown, 2010; Welton et al., 2009, 2010, in press). Although these recent efforts have shed light on cryptic diversity among Philippine vertebrates, the continued recognition of many widespread species may still compromise our understanding of patterns of regional diversity. Presently, the impressive diversity of endemic vertebrate species in the Philippines is recognized to be distributed among: (1) Pleistocene Aggregate Island Complexes (PAICs; Inger, 1954; Heaney, 1985; Voris, 2000; Brown and Diesmos, 2002), (2) individual islands within PAICs, and (3) upland subcenters of diversity within individual landmasses (review: Brown and Diesmos, 2009).

Historically, the shared body plans and similar external morphological features among populations of Brachymeles, and the absence of dense population sampling across the Philippines, proved problematic for diagnosing species (Brown, 1956; Brown and Rabor, 1967; Brown and Alcala, 1980). Although long considered to be a small clade of Southeast Asian lizards (the last revision enumerated only 15 species; Brown and Alcala, 1980), recent studies have significantly increased the known species diversity and expanded the range of variation in body form (Siler et al., 2009, 2009).

![Map of the Philippines showing the five recognized major Pleistocene Aggregate Island Complexes (PAICs) and additional deep-water islands. Current islands in the Philippines are shown in medium grey; light grey areas enclosed in black 120 m bathymetric contours indicate the hypothesized maximum extent of land during the mid-to late Pleistocene.](image-url)
Additionally, several rare, mid-to-high elevation species long represented by only a few specimens, in some cases without knowledge of their exact type locality (e.g., Brachymeles bicolor, B. elerae, B. wrighti, B. pathfinderi), have recently been rediscovered (Siler, 2010; Siler et al., in press; Siler and Brown, 2010). These studies, coupled with increased sampling throughout the Philippines, have provided a comprehensive data-set with which to begin evaluating the taxonomic stability of polytypic and widespread species across the Philippines. Additionally, the availability of tissue samples for all but two known species of Brachymeles now allows for robust estimates of phylogenetic relationships among recognized widespread and polytypic species, and evaluation of species boundaries. For example, Siler and Brown (2010) recently revised two polytypic species (B. buolengeri and B. schadenbergi) and one widespread species (B. talinis); this work resulted in the recognition of ten genetically and morphologically distinct species. Several other species including B. samarensis and B. boholensis (Brown, 1956; Brown and Rabor, 1967; Brown and Alcala, 1980) are still recognized as having widespread distributions that span multiple historically recognized biogeographic provinces in the Philippines (Dickerson et al., 1925; Kloss, 1929; Inger, 1954; Brown and Diesmos, 2002).

In this study we investigate the biogeography of Brachymeles from a phylogenetic perspective, providing the first estimate of phylogenetic relationships for this unique radiation of Southeast Asian lizards. We strove to estimate the phylogenetic position of Brachymeles among scincid lizards in order to provide insight into patterns of body form evolution and polarity of character change and provide the first statistical tests of several hypotheses. We provide the first glimpse into the major body form transitions in Brachymeles, particularly with respect to miniaturization, limb-reduction, and digit loss. We also employ a Bayesian ancestral area reconstruction to gain insight into the biogeographical history of the genus. Finally, we test the following taxonomic hypotheses: (1) Brachymeles is monophyletic; (2) All recognized and formerly recognized polytypic species are monophyletic; and (3) All recognized widespread species are monophyletic. Our data reveal patterns inconsistent with all of the above predictions and at odds with currently recognized taxonomy; we conclude that species diversity within the genus is vastly underestimated and that cryptic patterns of lineage diversification prevail in this poorly known group of Southeast Asian lizards.

2. Materials and methods

2.1. Taxon sampling and data collection

Ingroup sampling included 90 individuals collected from 43 localities, with 23 of the 25 currently recognized species of Brachymeles represented (Fig. 2; Appendix 1; Siler and Brown, 2010). The two missing species in our analyses are Brachymeles vermis and B. wrighti. Brachymeles wrighti is known from two damaged specimens from northern Luzon Island, and B. vermis occurs in the Sulu archipelago (where biologists are not permitted to work due to logistical and security obstacles). No tissues have ever been collected for either of these species.

Fig. 2. Distribution of pentadactyl and non-pentadactyl Brachymeles samples from the Philippines (see species keys within each map). Current islands in the Philippines are shown in medium grey; light grey areas enclosed in black 120 m bathymetric contours indicate the hypothesized maximum extent of land during the mid- to late Pleistocene.
To assess the monophyly of the genus as well as investigate appropriate outgroup taxa, a broad sampling of scincid species from the subfamilies Lygosominae and Scincinae were included, as well as a single outgroup sample from the family Lacertidae (Appendix 1). For all 108 samples, complete or partial sequences were collected for mitochondrial NADH Dehydrogenase Subunit 1 (ND1), NADH Dehydrogenase Subunit 2 (ND2), ATPase 8 (ATP8), and ATPase 6 (ATP6) genes (Table 1). Additionally, three nuclear loci, Brain-derived Neurotrophic Factor (BDNF), R35, and PTGER4, were completely sequenced for nearly all ingroup samples and many of the outgroup samples (Table 1, Appendix 2). All sequences were deposited in GenBank (Appendix 2).

Genomic DNA was extracted from liver tissues stored in 95–100% ethanol following a guanidine thiocyanate protocol (Esselstyn et al., 2008). We used a combination of published and newly developed primers, as well as a variety of thermal profiles for PCR products were purified with 100% ethanol following a guanidine thiocyanate protocol (Maddison, 2005). To assess phylogenetic congruence between the continuously gene sequences were assembled and edited using Sequencher 4.8 (Gene Codes Corp., Ann Arbor, MI).

### 2.2. Sequence alignment and phylogenetic analyses

Initial alignments were produced in Muscle (Edgar, 2004), and manual adjustments made in MacClade 4.08 (Maddison and Maddison, 2005). To assess phylogenetic congruence between the mitochondrial and nuclear data, we inferred the phylogeny for each gene independently using likelihood and Bayesian analyses, and performed pairwise partition homogeneity tests in PAUP 4.0b 10 (Swofford, 2002) with 100 replicates for each pairwise comparison to assess set congruence. Following the observation of no statistically significant incongruence between datasets, we felt justified in using the combined, concatenated, data for subsequent analyses. Exploratory analyses of the combined dataset of 108 individuals (including outgroup taxa with missing data for several genes) and a reduced dataset of individuals with no missing data exhibited identical relationships; we therefore chose to include all available data (108 individuals) for subsequent analyses of the concatenated dataset. Alignments and resulting topologies were deposited in TreeBase (SN11187).

 Parsimony analyses were conducted in PAUP* 4.0b 10 (Swofford, 2002), with gaps treated as missing data and all characters weighted equally. Most parsimonious trees were estimated using heuristic searches with 1000 random addition-sequence replicates and tree bisection and reconnection (TBR) branch swapping. To assess clade support, nonparametric bootstrapping was conducted using 1000 bootstrap replicates, each with 100 random addition-sequence replicates and TBR branch swapping.

Partitioned Bayesian analyses were conducted in MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003). The mitochondrial data set was partitioned by codon position for the protein-coding region of ND1 and ND2 and by gene region for the short gene regions ATP8 and ATP6. The Akaike Information Criterion (AIC), as implemented in jModeltest v1.0.1 (Guindon and Gascuel, 2003; Posada, 2008), was used to select the best model of nucleotide substitution for each partition (Table 2). The best-fit model for each data partition was implemented in subsequent Bayesian analyses. A rate multiplier model was used to allow substitution rates to vary among subsets, and default priors were used for all model parameters. We ran four independent Metropolis-coupled MCMC analyses, each with four chains and an incremental heating temperature of 0.05. All analyses were run for 18 million generations, sampling every 5000 generations. To assess stationarity, all sampled parameter values and log-likelihood scores from the cold Markov chain were plotted against generation time and compared among independent runs using Tracer v1.4 (Rambaut and Drummond, 2007). Finally, we plotted the cumulative and non-overlapping split frequencies of the 20 most variable nodes, and compared split frequencies among independent runs using Are We There Yet? [AWTY (Wilgenbusch et al., 2004)]. Although all samples showed patterns consistent with stationarity after 2.5 million generations (i.e., the first 12.5%), we conservatively discarded the first 20% of samples as burn-in.

In preliminary Bayesian analyses of the combined dataset, the independent runs failed to converge. We tried (1) lowering the incremental heating temperature to 0.02, (2) using an unconstrained branch length prior with an exponential distribution of 25 (Siler et al., 2010c; Marshall et al., 2006; Marshall, 2010), and (3) removing outgroup taxa with large amounts of missing data. Although some of the trials of individual permutations of parameters resulted in a failure to converge, the incorporation of the above, plus an unconstrained branch length prior with an exponential distribution and a mean of 25 resulted in convergence. Once complete convergence was achieved, we proceeded with final analyses, presented here.

### Table 1 Summary of primers and annealing temperatures employed in this study.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Primer name</th>
<th>Sequence</th>
<th>Annealing temperatures</th>
<th>Primer source</th>
</tr>
</thead>
<tbody>
<tr>
<td>NADH 1</td>
<td>16Sr tMet</td>
<td>5’-CTAGCTGATCTGATCACGCACCGGAC-3’</td>
<td>52–53°</td>
<td>Brandley et al. (2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5’-CTGGGTTATGGCGCCRCARAGCTT-3’</td>
<td>52–53°</td>
<td>Brandley et al. (2005)</td>
</tr>
<tr>
<td>NADH 2</td>
<td>ND2.Brach.F1</td>
<td>5’-TATCGCGAACAAAACACACC-3’</td>
<td>52–53°</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>ND2.Brach.R1</td>
<td>5’-ACGCGAGATTGATACCCG-3’</td>
<td>52–53°</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>ND2.Brach.R2</td>
<td>5’-GCCTCGATGGGTGCGTTTAC-3’</td>
<td>52–53°</td>
<td>This study</td>
</tr>
<tr>
<td>ATP8.6</td>
<td>ATP.R</td>
<td>5’-GCGCTTCTCTGCTTATAGTCGCT-3’</td>
<td>58°</td>
<td>Brandley (unpublished data)</td>
</tr>
<tr>
<td></td>
<td>ATP.F</td>
<td>5’-CTACGARACTTCGGGCGYAAATCACA-3’</td>
<td>58°</td>
<td>Brandley (unpublished data)</td>
</tr>
<tr>
<td>BDNF</td>
<td>BDNF.F</td>
<td>5’-CCCATGGAAGAAGTGAAGATCC-3’</td>
<td>55°</td>
<td>Crottini et al. (2009)</td>
</tr>
<tr>
<td></td>
<td>BDNF.R</td>
<td>5’-GCGCTTCTCTGCTTATAGTCGCT-3’</td>
<td>58°</td>
<td>Brandley (unpublished data)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5’-CCCATGGAAGAAGTGAAGATCC-3’</td>
<td>55°</td>
<td>Crottini et al. (2009)</td>
</tr>
<tr>
<td>PTGER4</td>
<td>PTGER4.F</td>
<td>5’-CCCATGGAAGAAGTGAAGATCC-3’</td>
<td>55°</td>
<td>Crottini et al. (2009)</td>
</tr>
<tr>
<td></td>
<td>PTGER4.R</td>
<td>5’-CCCATGGAAGAAGTGAAGATCC-3’</td>
<td>55°</td>
<td>Crottini et al. (2009)</td>
</tr>
<tr>
<td>R35</td>
<td>R35.F</td>
<td>5’-GCGCTTCTCTGCTTATAGTCGCT-3’</td>
<td>55°</td>
<td>Fry et al. (2006)</td>
</tr>
<tr>
<td></td>
<td>R35.R</td>
<td>5’-GCGCTTCTCTGCTTATAGTCGCT-3’</td>
<td>55°</td>
<td>Fry et al. (2006)</td>
</tr>
</tbody>
</table>
Partitioned maximum likelihood (ML) analyses were conducted in RAxMLHPC v7.0 (Stamatakis, 2006) on the concatenated dataset the same partitioning strategy as for Bayesian analysis. The more complex model ([GTR + I]) was used for all subsets (Table 2), and 100 replicate ML inferences were performed for each analysis. Each inference was initiated with a random starting tree, and employed the rapid hill-climbing algorithm (Stamatakis et al., 2007). Clade confidence was assessed with 100 bootstrap pseudoreplicates employing the rapid hill-climbing algorithm (Stamatakis et al., 2007).

2.5. Biogeographical reconstructions

To explore whether there is statistical support for historical biogeographic patterns within Philippine species of the genus *Brachymeles*, we compared empirically observed (extant) species distributions to estimates of ancestral distributions using the program BayesTraits version 1.0 (Pagel, 1994; Pagel and Lutzoni, 2002). For all analyses we examined a model of character evolution that assumed equal rates of distributional transitions. For all analyses we seeded the mean and variance of the gamma prior from distributions to estimates of ancestral distributions using the program BayesTraits version 1.0 (Pagel, 1994; Pagel and Lutzoni, 2002). For all analyses we examined a model of character evolution that assumed equal rates of distributional transitions. For all analyses we seeded the mean and variance of the gamma prior from distributions to estimates of ancestral distributions using the program BayesTraits version 1.0 (Pagel, 1994; Pagel and Lutzoni, 2002).
We enforced the ancestral states for all nodes sister to Philippine Brachymeles to be considered non-Philippine in distribution.

3. Results

3.1. Taxon sampling, data collection, and sequence alignment

The complete, aligned matrix contains 82 samples of Brachymeles, representing 23 of the 25 recognized taxa, for the mitochondrial genes and nuclear loci. Seventeen additional samples are included as outgroups, consisting of representatives from the subfamilies Lygosominae and Scincinae within the family Scincidae as well as a single representative from the lizard family Lacertidae. Following initial unrooted analyses, and the results of recent squamate evolutionary studies (Whiting et al., 2003; Townsend et al., 2004; Wiens et al., 2006; Brandley et al., 2005, 2008), we rooted the tree using samples of Takydromus sexilineatus from China. Within each gene, variable and parsimony-informative characters are observed as follows: 118 and 107 out of 158 (ATP8); 357 and 339 out of 683 (ATP6); 504 and 464 out of 966 (ND1); 575 and 531 out of 861 (ND2); 115 and 69 out of 715 (BDNF); 127 and 95 out of 490 (PTGER); 304 and 220 out of 689 (R35). The number of most parsimonious trees and consistency index (CI) resulting from MP analyses of the combined dataset is 78 trees (CI = 0.318).

3.2. Phylogenetic analyses

Analyses of the combined data (ND1, ND2, ATP8, ATP6, BDNF, PTGER4, R35) result in topologies with high Maximum Likelihood bootstrap support and posterior probabilities (Fig. 4). Topologies are congruent across these analyses (Fig. 4). All analyses support three clades of outgroup scincid taxa (Fig. 4). Outgroup samples from the subfamily Lygosominae are never recovered as part of a single clade (Fig. 4); however, given the possibility that the chosen root for analyses and the outgroup sampling strategy likely influence outgroup relationships, additional taxa should be obtained for a more exhaustive analysis of scincid relationships before definitive conclusions may be drawn.

The genera Eumeces and Plestiodon are recovered as a single, strongly supported clade in all analyses (Fig. 4, Clade 1). Although this clade is often supported as the closest group of outgroup species to Brachymeles + Davewakeum (ML; Fig. 4), support for its placement was always low, and in Bayesian analyses, the clade is regularly recovered as part of a three clade polytomy of outgroup samples (results not shown). The species Davewakeum miriamae is always recovered nestled within the genus Brachymeles between the clade B. apus + B. cf. apus from Borneo and all Philippine Brachymeles (Fig. 4).

Within the Philippines, all limbless species and the majority of limb-reduced species are recovered as part of two reciprocally monophyletic sub-clades, and together are sister to all pentadactyl species and the remaining non-pentadactyl taxa (Fig. 4, Clade 3). Sampled populations of the currently recognized widespread,
Limb-reduced species *Brachymeles bonitae* and *B. samarensis* are never recovered as monophyletic (Fig. 4, Clade 3). *Brachymeles cebuensis*, one of only two species of *Brachymeles* with unequal numbers of fingers and toes (three fingers/two toes) is recovered as sister to *B. samarensis* from Leyte Island (Fig. 4, Clade 3). Within Clade 1, all analyses recover a population of *Brachymeles bonitae*...
with one fore-limb digit and one hind limb digit as sister to the tri-
dactyl species \textit{B. tridactylus}, and the five other populations of \textit{B. bonitae} (Fig. 4). Additionally, a morphologically distinct popula-
tion of \textit{B. cf. bonitae} with limbs but no digits is supported to be the
sister species to \textit{B. tridactylus} (Fig. 4, Clade 3).

All pentadactyl species and three non-pentadactyl species of \textit{Brachymeles} are always recovered as part of a clade, sister to Clade 3 (Fig. 4, Clade 4). Although the two species formerly part of the \textit{Brachymeles schadenbergi} species complex (\textit{B. orientalis} and \textit{B. scha-
denbergi}) are always recovered as reciprocally monophyletic lin-
eages (Fig. 4, Clade 8), the polytypic species \textit{B. gracilis}, and the
four species formerly part of the \textit{B. boulenageri} species complex, are
strongly supported to be paraphyletic (Fig. 4, Clades 5, 6, 8).

All analyses recover four well-supported clades within Clade 4 (Fig. 4). Although Bayesian analyses provided high support for the relationships between these clades, likelihood analyses provide less support (Fig. 4, Clade 4).

Three non-pentadactyl species are recovered as part of Clade 4 in all analyses (Fig. 4). The tetradactyl species \textit{Brachymeles elerae} and the tridactyl species \textit{B. muntingkamay} are always recovered as sister taxa (Fig. 4, Clade 7); however, the relationship of Clade 7 to the other major clades in Clade 4 is less well supported. \textit{Brac-
hymeles pathfinderi}, the only other species with unequal digit num-
bers (five fingers/four toes), is recovered with strong support to be
nested within the polytypic species \textit{B. gracilis} (Fig. 4, Clade 6).

\textit{Brachymeles bicolor}, the longest species, is recovered as sister to a subclade of three medium-sized species (\textit{B. boulenageri} + \textit{B. min-
dorenis} + \textit{B. taylori}) and three large species (\textit{B. talinis} + two unde-
scribed species [Siler and Brown, 2010]), \textit{Brachymeles boholensis}, formerly recognized as a subspecies of the \textit{B. boulenageri} species complex, is consistently supported as part of Clade 8, sister to \textit{B. orientalis}, \textit{B. schadenbergi}, and \textit{B. makusog} (Fig. 4).

3.3. Topology tests

Results from the Bayesian methods and the approximately unbiased (AU) test were highly consistent. Among the taxonomy-
based hypotheses, both methods rejected hypotheses of mono-
phyly for the genus \textit{Brachymeles}, the widespread species \textit{B. bonitae},
\textit{B. gracilis}, and \textit{B. samarenis}, and the former polytypic species \textit{B. boulenageri} (Fig. 3).

Additionally, both methods failed to reject the monophyly of the former polytypic species \textit{B. schadenbergi}, now
recognized as two distinct sister species (Siler and Brown, 2010; Fig. 3). All biogeography-based hypotheses were rejected by both methods (Fig. 3).

3.4. Body form evolution

Our simplified mapping of major body plan variation onto the preferred phylogenetic tree (Fig. 4) makes it clear that the evolu-
tionary history of \textit{Brachymeles} involves multiple instances of evolu-
tionary shifts in body size, limb-reduction, and digit loss. Although
a full understanding of these trends will require a comprehensive
analysis of both external (body size, limb proportions, digital states)
and internal (vertebral numbers and elongation) morphology,
preliminary trends can be ascertained on the basis of results
presented here. If it is assumed, for example, that the ancestors
of \textit{Brachymeles} possessed similar body plans as the genus’ putative
closest relatives (\textit{Eumeces}, \textit{Pleistiodon}, \textit{Lygosoma}, \textit{Emoia Dasia} and
\textit{Eutrops}), then limb-reduction and loss may have occurred along the
lineages leading to \textit{B. apus} and \textit{B. cf. apus}, in the lineage leading to \textit{Davewakeima miriamae}, in the lineage leading to the \textit{B. bonitae} and \textit{B. samarenis} complexes, and independently in the lineages
leading to \textit{B. pathfinderi} and \textit{B. muntingkamay} + \textit{B. elerae}. It is interest-
ting to note that all of the known diversity of pentadactyl species
in the genus is endemic to the Philippine.

3.5. Historical biogeography

Although the placement of \textit{Brachymeles} within the family \textit{Scin-
cidae} remains somewhat ambiguous, the impact of ancestral range
for all \textit{Brachymeles} does not appear to heavily impact ancestral
reconstructions within the genus (not shown). The results of anal-
yses of ancestral areas are never significantly impacted by placing
reconstructions on the ancestral character states among outgroup taxa
and the node giving rise to all \textit{Brachymeles} (not shown). Without a
\textit{priori} knowledge of the true patterns of diversification within the
Philippines, we conservatively chose a model allowing for equal
rates of transition among major faunal regions of the Philippines.
Ancestral state reconstruction analyses resulted in many nodes
where the reconstructed ancestral range is ambiguous (Fig. 5). The
Luzon PAIC is the preferred ancestral range in most cases with
varying degrees of support (Fig. 5). Importantly, all analyses re-
sulted in unambiguous support for ancestral ranges at eleven
nodes in the phylogeny, supporting the Luzon, Negros-Panay, and
Mindanao PACS as the ancestral ranges for several clades of Phil-
ippine \textit{Brachymeles} (Fig. 5A–C). Given strong statistical support for
ancestral areas at eleven nodes, we are confident in hypothesis-
ning five dispersal events (Fig. 5), including clear dispersal from
Luzon to Mindoro, Luzon to the central Visayan islands, dispersal
from the Visayas to Mindoro and Luzon, and a clear instance of dis-
persal from the Mindanao island group to southern Luzon. Consid-
ering the moderate to strong statistical support for a specific
reconstruction at seven ambiguous nodes (indicated by Bayes fac-
tors of 4.13–9.10; Kass and Raftery, 1995; Nylander et al., 2004),
we infer the possibility of five additional dispersal events (arrows
not illustrated in Fig. 5).

4. Discussion

4.1. Taxon sampling

Our widespread sampling of species across the Philippines
allows for fine-scale resolution of phylogenetic relationships and
an unprecedented and comprehensive taxonomic review (Siler et al., 2009, 2010a,b; Siler and Brown, 2010; Siler et al., in press)

The two species missing in our analyses are \textit{Brachymeles wighti}
from northern Luzon Island and \textit{B. vermis} from the Sulu Archipel-
ago in the southern Philippines. Both species are represented by
only a few museum specimens worldwide, and no genetic samples
have ever been collected. Previous studies that have included sam-
pies of \textit{Brachymeles} have not been able to confidently place the
genus \textit{Brachymeles} within the family \textit{Scincidae} (Brandley et al.,
2005, 2008). Although our attempt to sample widely from out-
group taxa results in some well-supported relationships, the most
closely related species to the genus \textit{Brachymeles} remains unclear
(see also Brandley et al., 2005). We are unable to collect full se-
quence data for all included outgroup taxa (Appendix 2), and miss-
ing data may have contributed to weaker support for outgroup
relationships. It is anticipated that additional outgroup and gene
sampling will aid resolution of these relationships.

4.2. Phylogeny and cryptic genetic diversity

Cryptic diversity has been documented as a global phenomenon
(Pfenninger and Schwenn, 2007; Bickford et al., 2007), and we now
suspect the phenomenon to also characterize Philippine slender
skinks (Siler et al., 2009, 2010a,b; Siler and Brown, 2010; Siler et al., in press). Although we focus on diversity of skinks of the
genus \textit{Brachymeles}, our results support the taxonomic issues iden-
tified in numerous studies for the family \textit{Scincidae} (e.g., the non-
monophyly of \textit{Lygosominae}), and phylogenetic studies across the
family are needed to provide future taxonomic revisions. Although we did not expect to find that Davewakeum miriamae is nested within the genus Brachymeles, this result, in retrospect, is not surprising when comparing morphology of this genus to species of Brachymeles. Davewakeum shares several unique morphological features with B. apus, B. bonitae, and B. samarensis, including the fusion of the mental and first infralabial scales, the presence of a moderate-sized interparietal scale, nasal scales, two loreal scales, a frontal scale, frontoparietal scales, the presence of a single scale row on the lower eyelid (Siler et al., 2010b; Siler, pers. obs.), and a general elongated, limb-reduced body plan. With the results of this study, and the fact that the genus Brachymeles (Duméril and Bibron, 1839) was described well before Davewakeum (Heyer, 1972), we consider Davewakeum to be a junior synonym of Brachymeles and, consequently, Brachymeles miriamae, new combination, to be the fifth limbless species of Brachymeles. We note that at least one additional known limbless species (B. cf. apus, from Kalimantan, south Borneo) awaits description (Iskandar and Bickford, unpublished data).

With the conservative body plans within the genus Brachymeles have led to confusion over species boundaries, the long accepted recognition of polytypic species, and the recognition of “widespread” species with distributions across accepted faunal boundaries (Brown, 1956; Brown and Rabor, 1967; Brown and Alcala, 1980). With the exception of Brachymeles schadenbergi, the results of this study do not support the monophyly of the currently and previously-recognized polytypic species in the genus (Figs. 3 and 4). Additionally, all currently recognized subspecies are both strongly supported divergent lineages in the phylogeny and represent unique morphologies, most likely worthy of specific rank (Fig. 4; Siler and Brown, 2010; CDS, unpublished data).

The degree to which convergent morphology has led to the underestimation of diversity within the genus can further be exemplified by examining the “widespread” species densely sampled in this study. Populations of the previously-recognized widespread pentadactyl species, B. talinis, have recently been revised to represent five unique pentadactyl species, each with non-overlapping geographic distributions (Fig. 4): B. makusog (Siler et al., 2010a), B. talinis, B. sp. A (Siler and Brown, 2010), B. sp. B (Siler and Brown, 2010), and B. sp. C (Siler and Brown, 2010). Additionally, populations of the two former polytypic species, B. boulengeri and B. schadenbergi, have recently been revised, and all subspecies

![Fig. 5. Maximum clade credibility chronogram and estimated ancestral states of geographic range in Brachymeles skinks. Ancestral area reconstructions are indicated at each node. Triangles indicate unambiguous reconstructions of an ancestral area (posterior probability > 0.95), colored according to the hypothesized state (see key). Circles represent ambiguous character reconstructions, with colors representing the preferred area. Colored blocks at each ambiguous node represent alternate states recovered as possibilities in Bayes Traits analyses. Bayes factors are provided as an indication of the moderate to strong statistical support for preferred states at nodes with ambiguously reconstructed ancestral ranges. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)](image-url)
elevated to full, readily diagnosed species (Siler and Brown, 2010; Fig. 4, Clade 5, 8).

Currently, Brachymeles bonitae and B. samarensis are recognized to have atypical distributions that span multiple PAICs (Fig. 4, Clade 3). Brachymeles samarensis is known to occur on two islands in the Greater Luzon PAIC (Catanduanes, Luzon), as well as two islands and one small island group in the Mindanao PAIC (Leyte, Samar, Lapinig Island Group; Fig. 1 and 2). In comparison, B. bonitae has the widest recognized distribution of any species in the genus. Populations referable to this species occur in three distinct PAICs (Luzon, Mindoro, Negros-Panay) and two small deep-water island groups (Babuyan, Romblon). However, our results indicate that both B. bonitae and B. samarensis are complexes of numerous, morphologically similar species (Fig. 4, Clade 3). Although populations within both species complexes share similar body plans, unique sets of characters do exist, including differences in the numbers of fore- and hind limb digits (Siler and Brown, unpublished data).

These inter-population differences have historically been recognized as morphological variation within widespread species (Brown, 1956; Brown and Rabor, 1967; Brown and Alcala, 1980). However, upon our observation that character differences coincide with deep genetic divergences and biogeographic breaks in lineage distributions, we have begun to suspect that many inter-population variants in B. samarensis and B. bonitae will prove to be full species in accordance with any modern lineage-based species concept. Recently, taxonomic revisions such as this have been made for the B. boulengeri and B. shadengberi complexes (Siler and Brown, 2010).

Recent studies have revealed numerous other “widespread” Philippine endemic reptiles to actually represent complexes of cryptic species, with few species actually possessing distributions that span recognized faunistic boundaries (Brown et al., 2000; Brown and Diesmos, 2002, 2009; Siler et al., 2010a,b; Siler and Brown, 2010; Welton et al., 2009, 2010, in press). Exceptions do exist (Brown and Alcala, 1970), but many of these have turned out to represent invasive species with suspected histories of human mediated introductions (Diesmos et al., 2006; Brown et al., 2010). It is clear that the diversity of Brachymeles is vastly underestimated, and detailed morphological comparisons are needed to revise the taxonomy within the genus.

Finally, some level of uncertainty remains in the species diversity of Brachymeles outside of the Philippines (Fig. 4). The apparent disjoint distribution of Brachymeles in Southeast Asia may be an artifact of high levels of extinction outside of the Philippines or an absence of discovery. The phylogeny suggests that at least one genetically distinct, undescribed limbless species exists in southern Borneo (Kalimantan, Indonesia; Fig. 4), and it is highly probable that other undescribed species will eventually be discovered in other parts of Asia. It is noteworthy that the entire diversity of limbed, pentadactyl, reduced-limbed, and limbless body forms are found in the Philippines (Fig. 4); we take this pattern as a testament to the rich processes of diversification found within this small but remarkable island archipelago (Brown and Diesmos, 2009).

4.3. Diversification and body form evolution within a semi-fossorial genus

Previous surveys of body plan diversity within Brachymeles have focused solely on morphological variation (Brown and Rabor, 1967; Brown and Alcala, 1980, 1995), and have mentioned the potential for undocumented cryptic diversity within the genus as an ancillary possibility, not an expectation, and certainly not a prevailing phenomenon. However, a number of studies have shown that the evolution of a burrowing lifestyle is correlated with decreasing dispersal abilities (Selander et al., 1974; Patton and Yang, 1977; Patton and Feder, 1978) as well as changes in body form (see Crottini et al., 2009 for review). Several lineages of Brachymeles have undergone a reduction in limb size and digit numbers (Fig. 4), which may further reduce their vagility (Daniels et al., 2005; Mulvaney et al., 2005). Over time, reduced dispersal abilities may lead to an increasingly patchy distribution, reduction in gene flow among populations, and the accumulation of inter-population genetic differences (Nevo, 1979). This process also could be amplified within an island archipelago or a geographically complex island such as Luzon or Mindanao. We expect that this process has contributed to cryptic lineage diversification in this unique southeast Asian radiation.

The results of phylogenetic analyses provide evidence for three losses of external limb elements, and three distinct instances of changes in digit states. Although five species of Brachymeles are externally limbless (B. apus, B. minimus, B. miriamae, B. lukbani, B. vermis), it is currently unknown whether internal girdle elements are present in any of the five species, or if any of them retain some vestigial limb elements. Additionally, there is evidence for up to four independent losses of auricular openings, with the openings being completely covered by scales in B. apus, B. cf. apus, B. miriamae, all species of Brachymeles in Clade 3 of Figure ure4, and B. muntingkamy. It is also interesting to note that all species and populations with unequal digit numbers in the fore- and hind limbs have fewer toes than fingers in Brachymeles (B. bonitae, B. cebuensis, B. pathfinderi, B. samarensis), in contrast to many previous studies which have shown that reductions in digit number are more common in the fore-limbs of scincid lizards (Brandley et al., 2008; Skinner and Lee, 2010).

4.4. Biogeographic patterns

Although numerous previous studies have observed some phylogenetic patterns at least partially consistent with PAIC-based models of diversification (e.g., Heaney et al., 1998; Kennedy et al., 2000; Brown and Diesmos, 2002; Brown and Guttman, 2002; Evans et al., 2003; Esselstyn et al., 2004), we rejected all topologies predicted from a PAIC-based model (Fig. 3). Additionally, we rejected all hypotheses derived from patterns observed in other taxa (Alfaro et al., 2004; McGuire and Kiew, 2001; Siler et al., 2010c).

Phylogenetic analyses and ancestral state reconstructions provide support for multiple dispersal events in Brachymeles leading to complex and biogeographically convoluted distribution patterns observed today. Ancestral range reconstructions unambiguously estimate the ancestral range for 11 nodes in the chronogram, supporting a minimum of five geographic range shifts between major faunal regions in the Philippines (Fig. 3). Of these hypothesized inter-PAIC transitions, with the exception of a dispersal out of the Mindanao PAIC, all geographic transitions are hypothesized to have originated in the Luzon or central Visayan PAICs (Fig. 3A–C). Additionally we note that although only five inter-PAIC dispersal events are unambiguously reconstructed (Fig. 5A–C), the ancestral range for 5 additional nodes can be inferred with strong statistical support (Bayes factors 6–10; Bayes factors 6–10; Cass and Raftery, 1995; Nylander et al., 2004). Therefore, the possibility remains that additional instances of between-PAIC dispersal took place. Given that the major PAIC platforms of the archipelago have never been connected by dry land (Kloss, 1929; Inger, 1954; Heaney, 1985; Voris, 2000; Yumul et al., 2003, 2008), suggesting that faunal exchange among PAICs necessitates dispersal across ocean channels (review: Brown and Diesmos, 2009), we find it reasonable to conclude that much of the historical dispersion of Brachymeles throughout the archipelago has been through the process of walt dispersal over-water. Clearly the evolutionary and biogeographic history of semi-fossorial slender lizards has been heavily impacted by faunal exchange
throughout the archipelago. This may appear at odds with the general assumption of low vagility assumed for reduced-limbed lizards with a burrowing lifestyle, but we find it conceivable, and even plausible, that dispersal between islands is mediated by frequent rafting of mats of vegetation, topsoil, and logs; these possible vectors are frequently observed washing out of the mouths of rivers following heavy storms (CDS and RMB, personal observations).

5. Conclusion

Our study provides a comprehensive, phylogenetic analysis for a closely related group of lizards with a remarkable range of body from diversity. We have included samples from nearly all recognized species within the genus Brachymeles, and our intraspecific sampling has uncovered cryptic genetic diversity within many species (e.g., Brachymeles bonitae and B. samarensis). This study provides the foundation for a robust model system with which to address patterns of body form evolution, processes of diversification, and species delineation. With the exception of the recently published Lerista dataset (Skinner et al., 2008; Skinner, 2010; Skinner and Lee, 2010), our estimates of phylogeny represent one of the most comprehensive datasets for fine-scale studies of limb-reduction and loss in squamate reptiles.

It is clear that currently recognized diversity of skinks of the genus Brachymeles is vastly underestimated, and that numerous taxonomic revisions will be necessary to fully appreciate the processes of diversification within this nearly endemic Philippine radiation. New species await description (e.g., the new limbless species in southern Borneo; Fig. 4), and likely await discovery, and future survey work should focus on regions outside of the Philippines (e.g., Borneo, Malay Peninsula, Indochina). This study has revealed another case of extensive cryptic diversity in a once recognized assemblage of “widespread” Philippine species (Fig. 4). Together with numerous recent studies (Brown et al., 2009; Esselstyn et al., 2009; Esselstyn and Brown, 2009; Oliveros and Moyle, 2010; Siler et al., 2010c; Linkem et al., 2010b) this effort has resulted in wholesale discovery of numerous new species and cryptic evolutionary lineages of endemic Philippine vertebrates. Once considered a small radiation of Asian skinks, the recognized species diversity of the genus Brachymeles will likely increase by more than 300% over the next five years (Siler et al., unpublished data).

We rejected all PAIC-based models of diversification (e.g., Heaney et al., 1998; Kennedy et al., 2000; Brown and Diesmos, 2002; Brown and Gutmann, 2002; Evans et al., 2003; Esselstyn et al., 2004), as well as all patterns observed in other studies (Alfaro et al., 2004; McGuire and Kiew, 2001; Siler et al., 2010b; Fig. 3). However, the results of this study provide evidence for five major dispersal events across faunal zone boundaries that have given rise to the major clades of Brachymeles species diversity in the Philippines (Fig. 5). Surprisingly, all but one of these dispersal events are hypothesized to have originated in the Luzon or central Visayan PAICs (Fig. 3A–C). The results of this study, coupled with our knowledge of the geologic history of the region (Kloss, 1929, Inger, 1954; Heaney, 1985; Voris, 2000; Yamui et al., 2003, 2008), suggests that much of the historical faunal exchange of Brachymeles throughout the archipelago has been through the process of over-water (waif) dispersal. Without time-calibrated phylogenies, and the absence of closely related fossil calibrations, it is difficult to say when the hypothesized dispersal events occurred, leading to the complex distribution patterns observed today.

The transition from quadrupedal to limbless body plans has occurred repeatedly in independent lineages of squamate reptiles (i.e., snakes, lizards, amphisbaenians; Wiens and Slingluff, 2001; Greer, 1991; Pough et al., 2004). It is clear that these transitions also occur repeatedly within single radiations of closely related species (Fig. 4). The results of this study provide the first evidence of repeated limb, digit, and auricular opening loss in the genus Brachymeles. Given our results, and those of other studies that explicitly test morphological patterns of body form change within squamate reptiles (for review, see Wiens and Slingluff, 2001; Brandley et al., 2008), we are left with many unanswered questions. Given the apparent evidence for repeated body form change in Brachymeles, can we estimate the number of times characters have been lost (or potentially gained) in Brachymeles? Do the patterns of morphological changes observed within this unique radiation of Southeast Asian lizards support previous hypotheses of correlated morphological evolution associated with limb-reduction and loss in squamate reptiles (for review, see Wiens and Slingluff, 2001; Brandley et al., 2008)? Is there evidence for a gradual, evolutionary sequence involved in the process towards limb loss? What inferences can we make from statistical reconstructions (estimates) of ancestral morphology and character state change on the phylogeny? These and other broad-scale evolutionary questions that address the processes of body form evolution must be assessed within a comparative framework, and require the addition of robust morphological datasets.

Acknowledgments

We thank the Protected Areas and Wildlife Bureau (PAWB) of the Philippine Department of Environment and Natural Resources (DENR) for facilitating collecting and export permits necessary for this and related studies, wherein we are particularly grateful to M. Lim, J. Leon, C. Custodio, and A. Tagtag. Financial support for fieldwork for CDS was provided by several Panorama Fund grants from The University of Kansas Natural History Museum and Biodiversity Institute, a Madison and Lila Self Fellowship from the University of Kansas, Fulbright and Fulbright-Hayes Fellowships, as well as an NSF doctoral dissertation improvement grant (DEB 0804115 to CDS) and NSF EF-0334852 and DEB 0743491 to RMB. For the loans of specimens (museum abbreviations follow Leviton et al., 1985), we thank J. Vindum and A. Leviton (CAS), R. Sison and V. S. Palpal-lato (PNM), J. F. Rina (CMNH), A. Resetar and H. Voris (FMNH), R. Crombie (USNM), T. LaDuc (TNHC), D. Bickford (RMRB), L. Grismer (LSUHC), and M. Lakim of Sabah Parks (SP). We are appreciative of critical reviews of the manuscript provided by D. Blackburn, J. Esselstyn, M. Holder, D. McLeod, J. Oaks, and two anonymous reviewers. We thank the CAS’s Stearns Fellowship and the MCZ’s Ernst Mayr Fellowship for funding multiple visits to examine comparative material, and CDS and RMB thank E. Cunanan and M. Diesmos for their support and hospitality during numerous visits to the Philippines.

Appendix A. Supplementary data


References


